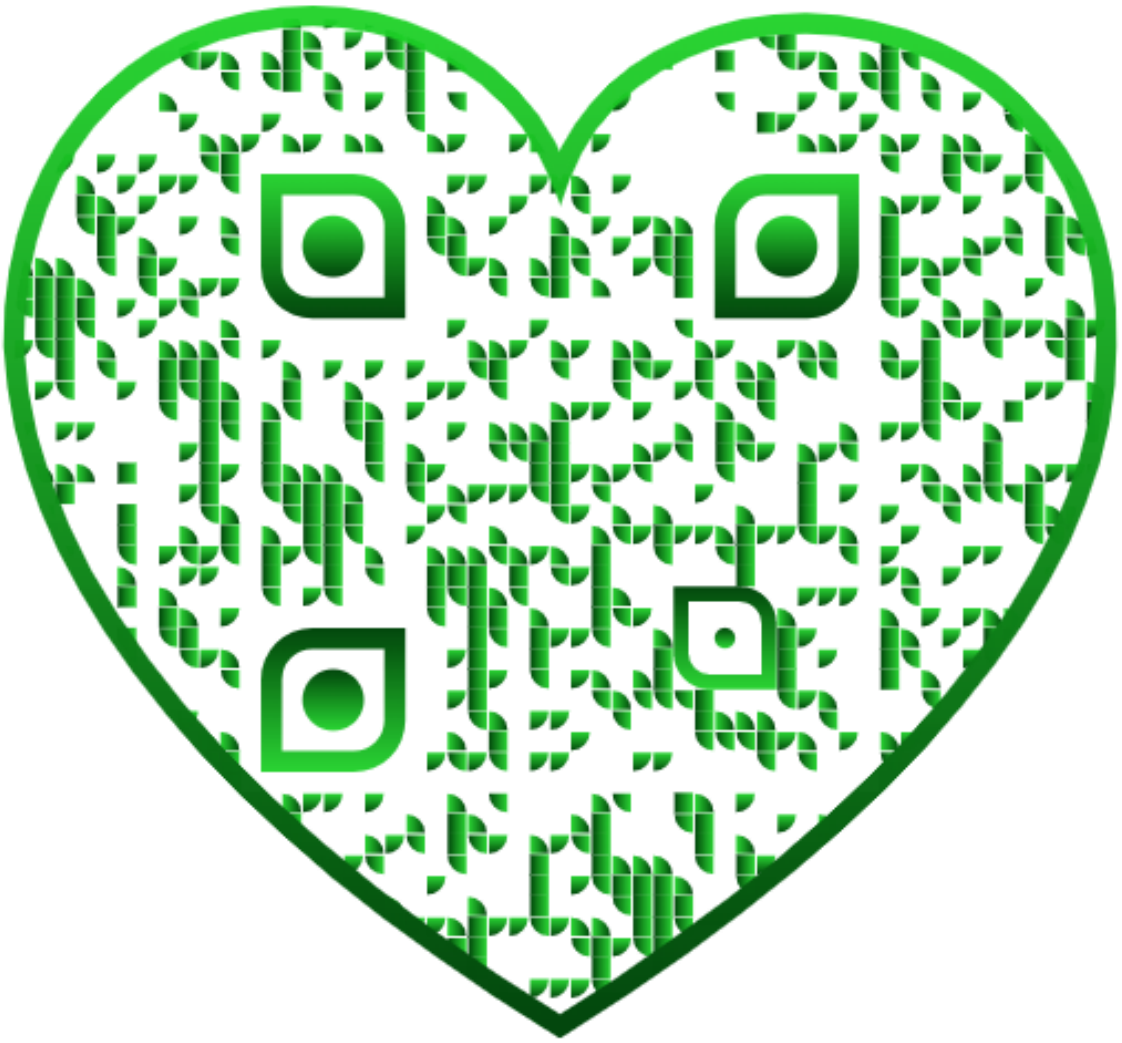
Pharmaceutical Analysis I (Phar3121)

Course Objectives

- After completing this course, students will be able to understand
  - the importance of quality control,
  - the purpose of pharmacopoeia,
  - the steps involved in drug analysis,
  - the sample pretreatment,
  - different analytical methods and their use in pharmaceutical analysis,
  - They will also able to carry out analysis for different pharmaceuticals and interpret and report data



1. INTRODUCTION TO PHARMACEUTICAL ANALYSIS (18 HR)

By

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1. Introduction

Pharmaceutical analysis:

- It is a science which deals with ***identification, characterization and quantification*** of drugs in raw materials, dosage forms and biological fluids or
- It is an applied science that ensures the ***safety, efficacy and stability*** of pharmaceutical products by using physical, chemical, biological methods; and
- It is also a technique that is used in ***elucidation*** of drug entities from natural products.

1. Introduction …

- Generally, pharmaceutical analysis procedures may be used to answer any of the questions below:
  - Do the ***identity and purity*** of a pure drug substance meet specification?
  - What is the ***percentage of the stated content*** of a drug present in a formulation?
  - What is the ***stability*** of a drug in the formulation and hence the shelf–life of the product?
  - Do the identity and purity of ***excipients*** meet specification?

1. Introduction …

- **Scope (areas) of pharmaceutical (drug) analysis:**
  - **Pharmaceutical industry**
    - ✓ *Raw material control*
    - ✓ *In-process control*
    - ✓ *Dosage form control*
  - **Government drug control laboratory (regulatory agencies)**
    - » *Manufacture lab. Procedure control*
    - » *Products control*

1. Introduction …

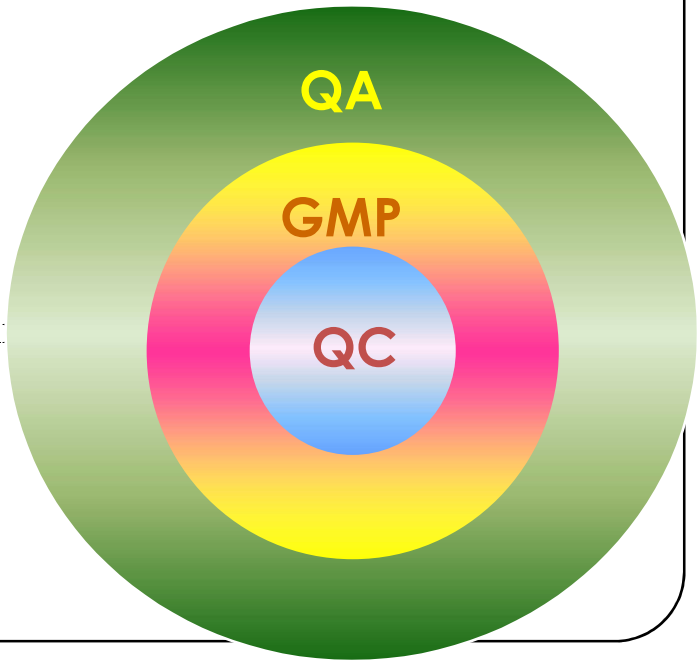
- **Pharmaceutical research labs**
  - Separation, Identification, Quantification, Molecular characterization
- **Laboratories requiring pharmaceutical analysis:**
  - Government regulatory agencies
  - Manufacture of raw materials for drugs
  - University and other non-commercial research centers

1.1. Introduction to quality control and Quality Assurance

- **Quality:**
  - A measure of a product's or service's ability to satisfy the customer's stated or implied needs.
  - In the pharmaceutical industry represents both products and process.

"Fitness for purpose"

- When the medicine is given to patients, it must have been appropriately manufactured, tested and packaged to assure that:
  - It is the correct product,
  - It is the correct strength,
  - It has not degraded,
  - It is free from harmful impurities and micro-organ
  - It is correctly labeled and
  - It is properly sealed in a suitable container



- **Quality Control (QC):**
  - The operational techniques and activities that are used to *Check or test the specifications are met* *OR*
  - Steps taken during the generation of a product or service to ensure that it meets requirements and that the product or service is **reproducible**.
- ***Quality assurance (QA):*** *The sum total of the organized arrangements made* with the objective **of ensuring that all materials are of the quality required for their intended use and that quality systems are maintained.**
- **Quality control can be expressed as internal and external**
  - ***Internal QC*** refers to all measures taken to achieve precision and accuracy in the laboratory
  - ***External QC*** compares and measures the performance of different laboratory

- **Quality control is also concerned with**
  - ***sampling, specification and testing*** with the organization documentation and release procedures which insure that the necessary and relevant tests are actually carried out.
  - ***no products released for sale or supply*** until their quality has been judged to be satisfactory.
- **Good manufacturing practice (GMP)**:
  - *GMP is that part of QA which ensures* products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization or product specification**.**

- **The tests of quality control may belong to the following types:**
  - ✓ Physico-chemical methods
  - ✓ Microbiological methods
  - ✓ Biological methods

❖ **In conclusion, end-product testing is not adequate, Why?**

❖ By QA, it means to include all those aspects which start with the procurement of raw materials to the finished product available at the medical store until it gets consumed by the patient.

❖ In robust modern quality systems:

"***Quality should be built into the synstem.***

1.1. Introduction to quality control….

- ***There is a common theme to all quality systems whether used for pharmaceutical production or not.***
  - **The quality system must be described in written documents approved by management (policies, quality manual, etc).**
  - **The quality system must be regularly reviewed.**
  - **All operations that can effect quality must be described in written and approved procedures (standard operating procedures, SOPs).**
  - **Materials must be appropriately approved prior to use.**
  - **Output (product) must be appropriately inspected prior to release.**

1.1. Introduction to quality control….

- **Equipment used must be fit for purpose (qualified/validated, calibrated and maintained).**
- **Personnel must be trained in the quality system and in operations they perform.**
- **Written records must be kept to demonstrate quality procedures have been followed.**
- **There must be regular internal quality audits to ensure quality is maintained.**

1.1. Introduction to quality control….

Objectives of Quality Control

- Make sure that proper sampling and analytical test are done.
- Make sure that the finished products contain **active ingredients** complying with the **qualitative and quantitative composition** and enclosed with in their **proper container** and **correctly labeled.**
- Make sure that **no batch of product** is released to sale or supply prior to certified
- In conclusion, all quality systems are to do with people, materials, equipment, records and procedures.
  - ***Generally speaking Objectives of Quality Control:-to render good of quality products and/or service to the customer.***

1.1.1.QC procedures in pharmaceutical industries

- The main areas of QC procedures include the various steps to ascertain that within reasonable limits the drug should have the following characteristics:

1. Genuine quality and purity

- **Identification test**: *should be performed to ensure the pharmaceutical product to be genuine*
  - *Usually an IR spectrum of a reference or authentic compound is utilized to be compared with.*

1.1.1.Control procedures.....

- **Purity:** *the drug should be physically and chemically pure.*
  - *Purity is the state of a chemical compound when no impurity can be detected*
- The purity of pharmaceuticals can be ascertained through purity test.
  - ✓ The tests for purity involve tests for the presence of impurity and fix the limits of tolerance for these impurities.
- Some of the tests which may be run to ascertain the purity of substances are:

i. colour, odour and taste tests

- They are used when other tests of purity are not available,
- They have limited value, but determine whether a substance is reasonably pure or not

1.1.1.Control procedures…..

ii. Determination of Physico -chemical constants

- This ensures whether the substances are reasonably free from other substances, although they fail to indicate the nature of impurities present.
- ***Solubility*** of substances in different solvents, determination of ***mpt. and bpt., optical rotation and refractive index*** have reliable values which can reveal the purity of the substances.

❖ **mpt.** and **bpt.** are usually expressed in ranges to define purity

E.g. –Metronidazole, MP: 159-162 °C.

❖ **Specific optical rotation-**

E.g. Ergometrine: +50 to +56° (1% w/v solution in water)

Phenylepherine: -46 to -47.5° (2% w/v solution in water)

1.1.1.Control procedures…..

iii. Determinations using analytical techniques

- Identification of drug purity can be accomplished by utilizing simple to more sophisticated analytical methods. It includes:
  - ✓ Titrimetric techniques - Acidimetry and alkalinimetry, Non-aqueous titrations
    - Karl- Fischer titrations, Gravimetric titrations
  - ✓ Separation techniques - Chromatographic methods (TLC, GC, HPLC, etc.)
    - Hyphenated techniques (GC-MS, HPLC-MS. etc.)
  - ✓ Spectroscopic techniques - UV-Visible, NMR, IR, MS. etc.
- These techniques *are* very sensitive to control impurities in very minute conc.

1.1.1.Control procedures…..

iv. Miscellaneous characteristics

- A large number of characteristics still need to be monitored to ascertain the purity and authenticity of drugs. These include:

Sulfated ash:

- ✓ used to test synthetic organic compounds
- ✓ Determined by ignition with conc. sulphuric acid; metals thus remain as sulphides that are stable to heat
  - E.g. Ascorbic acid — sulphated ash should not be more than 0.1%

Loss on drying:

- ✓ refers to the net weight of a pharmaceutical substances being dried at a specified T

E.g. Aspirin: for 1g sample when dried (under reduced pressure over silica gel) for 5hrs. It should not loss more than 0.5% of its weight.

1.1.1.Control procedures.....

2. A drug should contain the same amount of active ingredients as stated on the label.

- This is achieved through:
  - assay of active ingredients/finished product
  - dosage form uniformity, content uniformity and weight variation
- The standards of pharmaceutical chemicals and dosage forms should fulfill the criteria set by various official compendia.
- These include:
  - highest attainable standards for chemical purity; there may be differences in methods of manufacture and changing pattern of stability.

E.g. Chloramphenicol: 98-102%

Acetylsalicylic acid: 99.5-100.5%

3. A drug should retain quality in terms of shelf-life and stability

- **Stability** is defined as the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, therapeutic and toxicological specification.
- **Shelf-life** is the period at which the drug remains to its 90%.
- Stability indicating assay is the assessment of a pharmaceutical dosage form (excipients and active ingredients) under stressful conditions

➢ There are factors which can contribute to instability of a pharmaceutical product

a. Incompatibility:

Instability of a pharmaceutical product due to **undesired reaction** between two or more components of the product.

1.1.1.Control procedures.....

- The undesired reaction may lead to:
  - ✓ Physical instability: physical changes (colour, odour, gross precipitation)
  - ✓ Chemical instability: identified by chemical analysis.
  - ✓ Therapeutic incompatibility: leads to excessive potentiation or decrease effectiveness.

b. Oxidation/reduction reaction, hydrolysis, …

- ✓ Influenced by temperature, radiation, catalysis, moisture, etc.

c. Photochemical reactions

- ✓ Light can cause degradation thus colored glass containers are used

"Hence these all factors should be controlled in the production process, raw material as well as upon storage of the finished product as they do have influence on the shelf-life as well"

1.1.1.Control procedures.....

4. A drug should not contain undesirable impurities

- **Impurities:** are defined as undesired or extraneous materials in a given substance.
- In pharmaceuticals, impurities can be defined as **unwanted chemicals** that remain with the API or developed during formulation or upon aging of the active ingredient (dosage form) that arose in either toxicity or decrease or increase potency.
- The presence of impurities even in small amount may influence efficacy and safety of pharmaceutical products.
- ✓ There are two types of impurities:

1. ***Impurities associated with the API***
2. ***Impurities created during formulation and with aging***

1.1.1.Control procedures.....

- According to ICH guidelines, impurities associated with APIs are classified into the following categories:

A. Organic impurities (process and drug-related):

- May arise during the manufacturing process and/or storage of the drug substance, include the following:

i. Starting materials or intermediates:

- the most common impurities found in every API.

Eg. In **paracetamol bulk,** there is a limit test for ***p-aminophenol,*** which could be a starting material for one manufacturer or be an intermediate for another.

1.1.1.Control procedures.....

***ii. By-products*:**

In the case of paracetamol bulk, diacetylated paracetamol is the by product

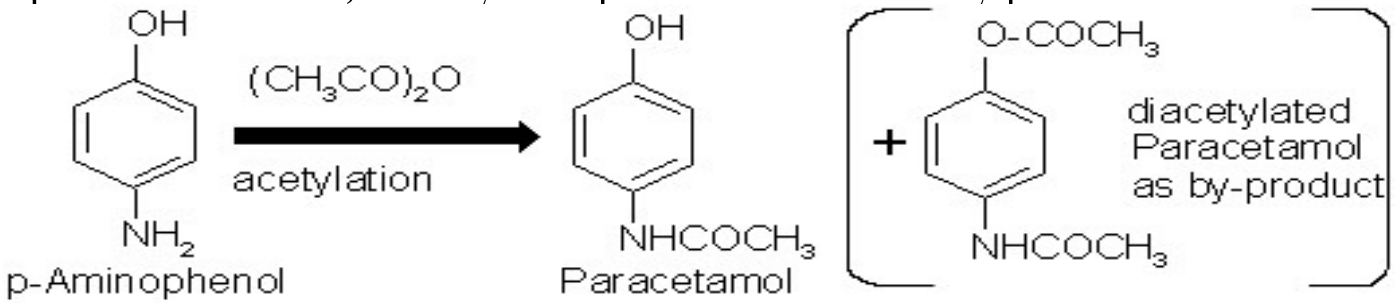


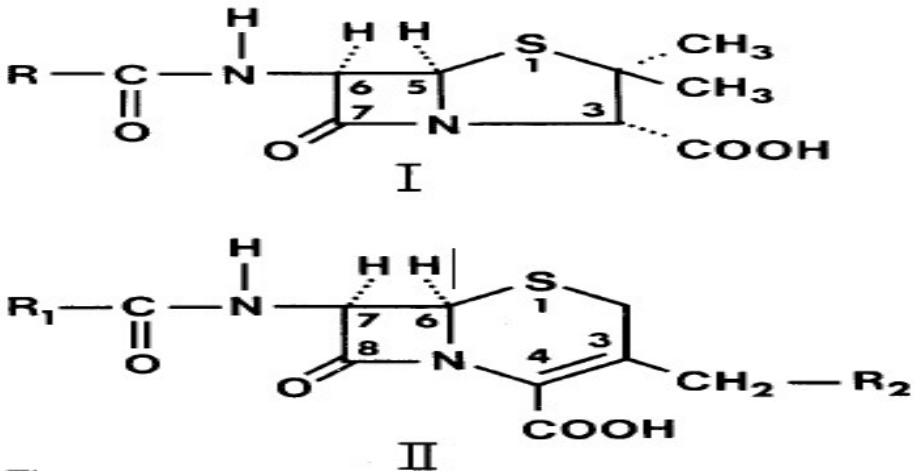
Fig. Production of Paracetamol from intermediate, p-Aminophenol.

***iii. Degradation products*:** degradation products resulting from the instability of API or unfavorable storage condition.

Eg. The degradation of penicillins and cephalosporins

- ✓ The presence of a ß-lactam ring as well as that of an α-amino group in the C6/C7 side chain plays a critical role in their degradation

1.1.1.Control procedures…..



iv. Reagents: These chemicals are less commonly found in APIs.

"In general, an individual API may contain all of the above-mentioned types of organic impurities at levels varying from negligible to significant"

1.1.1.Control procedures.....

B. Inorganic impurities:

- Inorganic impurities may also derive from the manufacturing processes used for bulk drugs (API).

- They are normally known and identified and include the following:
  - ✓ ***Reagents, ligands, and catalysts*** - The chances of having these impurities are rare
  - ✓ ***Heavy metals*** - The main sources of heavy metals are the water used in the processes; can be easily avoided using demineralized water
  - ✓ ***Other materials*** (eg, filter aids, charcoal):

1.1.1.Control procedures…..

C. Solvent residues:

- are organic volatile chemicals used during the manufacturing process or generated during the production.
- ❖ It is very difficult to remove these solvents completely by the work-up process; however, efforts should be taken to the extent possible to meet the safety data.
- ❖ Depending on the possible risk to human health, residual solvents are divided into 3 classes.

Class I solvents:

- **Solvents to be avoided**: known to cause unacceptable toxicities, strongly suspected human carcinogens
  - ✓ such as benzene (2 ppm limit) and CCl_4 (4 ppm limit) have to be avoided.

1.1.1.Control procedures…..

Class II solvents:

- ✓ **Solvents to be limited**: known non-genotoxic, animal carcinogens or are possible causative agents of irreversible toxicity
- ✓ such as methylene chloride (600 ppm), methanol (3000 ppm), pyridine (200 ppm), toluene (890 ppm), N,N-dimethylformamide (880 ppm), and acetonitrile (410 ppm).

Class III solvents:

- ✓ have permitted daily exposures of 50 mg or less.
- ✓ acetic acid, acetone, isopropyl alcohol, butanol, ethanol, and ethyl acetate

1.1.1.Control procedures.....

Impurities related to formulation

- Impurity formed during formulation

a) Method related

- A known impurity, 1-(2,6-diclorophenyl)indolin-2-one is formed in the production of a parenteral dosage form of diclofenac sodium if it is terminally sterilized by autoclave.

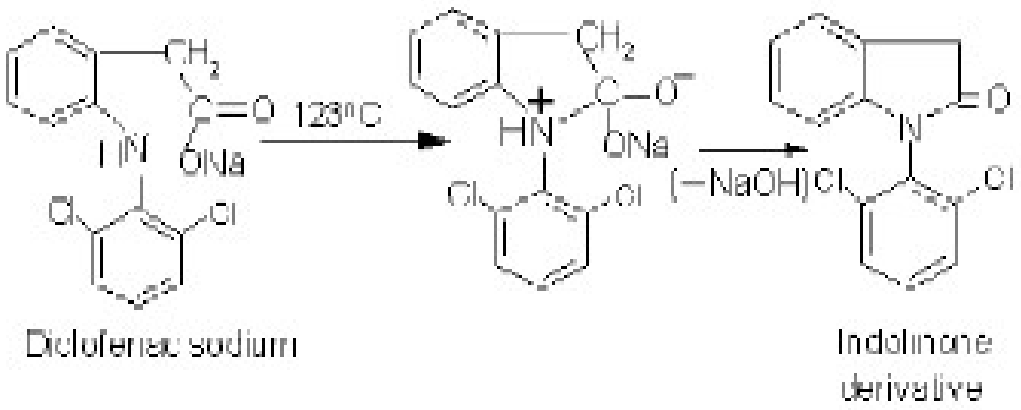


Fig. Formation of impurity on autoclaving of Diclofenac sodium

1.1.1.Control procedures.....

b) Environmental related

- Environmental factors that can reduce stability include the following:

Exposures to adverse temperatures-

- ✓ Eg. Vitamins as drug substances are very heat-sensitive and degradation frequently leads to loss of potency, especially in liquid formulations.

Light-especially UV light –

- ✓ Eg. Ergometrine as well as methyl ergometrine injection is unstable under tropical conditions such as light and heat.

Humidity-

- ✓ For hygroscopic products, humidity is considered detrimental to both bulk powder and formulated solid dosage forms. Aspirin and ranitidine are classical examples.

1.1.1.Control procedures…..

c) Dosage form factors related

- In general, liquid dosage forms are very much susceptible to both degradation and microbiological contamination.
- In this regard,
  - water content,
  - pH of the solution/suspension,
  - compatibility of anions and cations,
  - mutual interactions of ingredients, and the primary container are critical factors.

1.1.1.Control procedures.....

Formation of impurities on aging

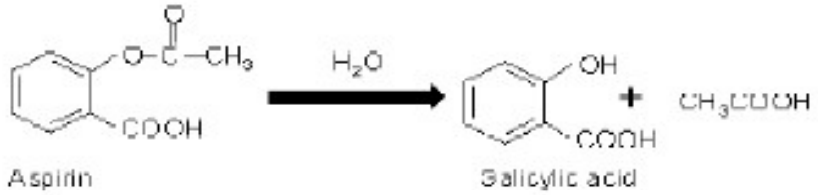
a) Mutual interaction amongst ingredients

- ✓ Most vitamins are very labile and on aging they cause a problem of instability in different dosage forms, especially in liquid dosage forms.
- ✓ Degradation of vitamins such as folic acid, pantothenic acid, cyanocobalamin, and thiamine do not give toxic impurities;
- ✓ however, potency of active ingredients drops below pharmacopoeias specifications.

1.1.1.Control procedures.....

b) Functional group-related typical degradation

Hydrolysis -Examples included the following: Aspirin, benzocaine, cefotaxime, cocaine… especially in liquid dosage forms.



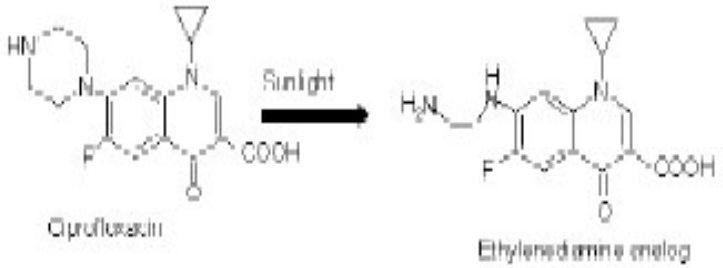
Oxidative degradation

- ✓ hydroxyl group directly bonded to an aromatic ring (eg, phenol derivatives such as catecholamines and morphine),
- ✓ conjugated dienes (eg, vitamin A and unsaturated free fatty acids),
- ✓ heterocyclic aromatic rings, nitroso and nitrite derivatives, and aldehydes (eg, flavorings) are all susceptible to oxidative degradation.

1.1.1.Control procedures.....

Photolytic cleavage - Pharmaceutical products are exposed to light while being manufactured as a solid or solution, packaged, held in pharmacy shops or hospitals pending use, or held by the consumer pending use

Eg. In ciprofloxacin eye drops preparation (0.3%), sunlight induce photo cleavage



Ciprofloxacin Ethylenediamine analog

Decarboxylation- Some dissolved carboxylic acids lose carbon dioxide from the carboxyl group when heated.

Eg. >> p-aminosalicylic acid

- **Generally, Sources of Impurities in Drug Products can be:**

1. Raw materials used in the manufacture

2. Method or the process used by the manufacturer

   - Reagents, Solvents, Reaction vessels, Catalysts used in the process,

3. Due to the instability of the product

4. From atmospheric contaminates:- dust(aluminum oxide, silica, sulfur, etc), sulfur dioxide, hydrogen sulfides, arsenic

5. Manufacturing hazards

   -microbial contamination, packing errors,

6. Storage conditions- Temperature effects, Physical changes

1.2. COMPENDIA

1.2 Compendia

- It is a collection of concise but detailed information about a drug.
- Pharmacopeias and formularies are books of standards for pharmaceuticals and medical devices containing **specifications** and **procedures of tests.**
- They are collectively referred as the drug compendia.
- Published by the authority of a government or a medical or pharmaceutical society.
- The main objective of the compendia:
  - ***To control the quality of medicine as pre standard***
  - ***To ensure the public health***
  - ***To support the availability of safe, effective, good quality pharmaceutical care for all***
- Drug compendia are sub classified as **official and non-official**

1. Official compendia

- It is a collection of list of drugs and devices which have been recognized as **legal standards of purity, quality and strength** that is accepted by some recognized authority.

Pharmacopeia

- It is a book containing a list of medicinal substances, their dosage forms or devices with **descriptions of test methods, specifications for purity and strength** accepted by some recognized authority.
  - The recognized authority which issue the books of standards in most countries is the government
  - In some countries e.g. USA both the national pharmacopoeia and national formularies are published by a private non profit making organizations

- Components and **specification** requirements of pharmaceutical dosages:

Excipients: therapeutically inactive but have indirect effect (like the rate of release (dissolution and disintegration) of the medicament.

- They should be safe(non toxic), not having any interference with the therapeutic activity and analytical procedure

Active components: therapeutically active form of a drug.

- They should have the highest attainable standard in terms of chemical purity and biological response

Some specification requirements for pure drugs (API):

- Description: Crystalline, amorphous, etc.
- Solubility
- Identification tests
- Physical constants
- Limit tests for classically well known toxicants
- Assay
- Limit of degradation products

The compendia (cont…)

Specification requirements of dosage forms:

- Identification
- Assay (with reference to label claim)
- Disintegration
- Dissolution
- Content uniformity tests
- Packaging and storage

The compendia (cont…)

The following are some of the representatives:

- United States Pharmacopeia and National formulary (USP and NF)
- British Pharmacopoeia (BP)
- International Pharmacopoeia (PhI)
- European Pharmacopoeia (Ph. Eur.)
- Indian Pharmacopeia (IP), etc

- **Pharmacopeias or formularies are generally national in origin and scope**

The compendia (cont…)

2. Non official compendia

- These are secondary reference sources that are not subject to categories of official compendia. They include treatises, monographs and text books.

- **Treatise:** it is a comprehensive, exhaustive, systematic or critical approach to a broad topic or whole field of knowledge.
  - ✓ It is generally written for specialist in a given field.

- ✓ **Monograph:** is a work of writing upon a single subject, usually by a single author.
  - ✓ It is by definition a single document that forms a complete text in itself

The compendia (cont…)

- **Text books:** they concentrate on principle rather than on details of the last minute (very latest) development in the field.
- **Martindale (Extrapharmacopeia)**- is based on published information
  - ✓ it is not a book of standard hence the inclusion of a substance or preparation is neither to be considered as a recommendation for use nor confer any status on substance or preparation.
  - ✓ Its main objectives are to provide in practicing pharmacists and physicians with unbiased evaluated information on drugs and medicaments (proprietary preparations) used through out the world

Components of pharmacopoeia

a) General notices:

- General notices provide the overall guiding principles for using the monographs and general chapters.
- It mainly clarifies information provided in the pharmacopoeia to the reader.

EXAMPLES

- **Tolerances:** e.g. Aspirin:-should have not less than 99.5% and not more than 100.5% of the label claim. Reason for tolerances:
  - Assay errors
  - Basic compounding or manufacturing errors
  - Detection of chemical substances

The compendia (cont…)

- **Tests and assays:**
  - ✓ ***About:*** approximately (fairly correct or accurate; near to the actual value).
  - ✓ ***Exactly:*** implies an error of 0.1% in the case of weighing or 0.05ml in a 50.0ml burette
  - ✓ ***Corresponds***: similar or equivalent in character or quantity.

The compendia (cont…)

- ***water bath***' means a bath of boiling water, unless water at some other temperature is indicated in the text
- ***Drying to constant weight***: means that drying shall be continued until two consecutive weighing do not differ by more than 0.5mg per gram and weighing should be 1hr apart.
- ***Blank determination***: conducted in exactly same manner as in the assay or test except that there is no sample.
- ***Indicators*****:** implies usually approximately 0.2ml or 3 drops of indicators solution

The compendia (cont…)

- **Packaging, storage and labeling:**
  - ✓ The container should not interact physically or chemically with the chemical placed in it
  - ✓ The **container** should be
    - ***Light sensitive container:*** which does not transmit more than 18% of UV radiant energy falling up on it.
    - ***Well-closed container***: protects contents from extraneous solids and from loss of drug under ambient conditions.
    - ***Tightly closed container:*** protects contents from contamination by extraneous liquids, solids or vapors or from loss of drug due to evaporation.
    - ***Tamper*** *–Evident Packing, airtight container, Single–dose container* etc

The compendia (cont...)

Storage conditions

- ✓ ***Cold*:** any T^O not exceeding 8 ^OC and usually between 2^O and 8^OC.
- ✓ ***Cool*:** Any T^O between 8^O and 25^OC.
- ✓ ***Room Temperature***: T^O prevailing in working area
- ✓ ***Warm***: Any T^O between 20^O and 40^OC.
- ✓ ***Excessive heat***: Any To above 40^OC.

- Where no specific conditions are indicated it is to be understood that storage conditions includes protection from moisture, freezing and excessive heat.

The compendia (cont…)

- Other provisions applied to general chapters and monographs
  - ✓ Quantities
  - ✓ Apparatus and procedures
  - ✓ Drying and ignition to constant mass
  - ✓ Reagents
  - ✓ Solvents
  - ✓ Expression of content
  - ✓ Temperature
  - ✓ Abbreviation and symbols
  - ✓ Units of the international system (SI) used in the pharmacopoeia and equivalence with other units

The compendia (cont…)

b) Monograph:

- A detailed and documented article on a particular subject.
- A statement that specifies
  - the kinds and amounts of ingredients a drug or class of drugs may contain,
  - the directions for the drug's use,
  - the conditions in which it may be used, and the contraindications to its use.
- It also defines qualitative and quantitative characteristics with the test procedures and their acceptance limits.

The compendia (cont…)

- **Official monograph generally** is both descriptive and informative: **includes the following**

1. **Title** : generic name, molecular structure, formula and weights are given, Chemical names have also been provided
2. **Therapeutic category**
3. **A description** of its physical characteristics
4. A statement of the **minimum standard of purity** as determined by the assay
5. **Identification tests** for use in verifying the identity of the product
6. **limit tests** to exclude excessive contamination and /or decomposition

The compendia (cont…)

7. **Assay method**: official quantitative procedures for determination of the active ingredient, solvents and other constituents require to asses compliance with the standard
8. **Physical Constants** and tests which supplement the standard
9. Other information on:
    - packaging and storage conditions
    - labeling and other regulatory requirements
    - Dosage
    - cautionary notices on cytotoxic and other such dangerous materials

The compendia (cont…)

General tests

- Contain information basic test procedures which are needed as general requirements to be tested for the generalized dosage form (tablet, injection, capsules, suppositories, etc.). It includes
  - Apparatus for tests and assay
  - Physical tests and determinations : - Disintegration, Dissolution, Color, Loss on drying, Refractive index, Optical rotation
  - Chemical tests and assays :
    - Identification tests, Limit tests and assays
    - Nitrogen determination, Water determination
    - Vitamins assay

The compendia (cont…)

- **Microbial tests**
  - Antimicrobial preservative effectiveness tests
  - Microbial limit tests
- **Biological tests and assays**
  - Antibiotic assay
  - Bacterial endotoxin tests
- **Limit tests**
  - The substances that are used in pharmaceutical field should be almost pure so that they can be used safely.

The compendia (cont…)

- It is almost impossible to get an absolutely pure material as impurities get incorporated in to them either during manufacture, purification or storage

- The total impurities should be in certain minimal range, i.e. there should be acceptance criteria for specified impurities.
- In general, specification limit not more than 0.1% for any unspecified impurity should be included.
- Limit tests are designed to identify and control small quantities of impurity which are likely to be present in the substance

The Law and pharmaceutical analysis

Law – is the body of principles that govern conduct and observance of which can be enforced in courts.

- It can distinguish **what is permissible from what is not**.

- **Food, Medicine and Health care Administration and Control Authority of Ethiopia (FMHACA)**

- FMHACA of Ethiopia is a federal agency established by Proclamation No. 661/2009.

The Law and pharmace…

Mission

- To promote and protect public health by ensuring the safety, efficacy, and quality as well as the proper use of the drugs
- FMHACA of Ethiopia has four major Departments:
  1. Food, Drug Evaluation and Registration Department (DERD)
  2. Planning, Drug Information Establishment and Distribution Department (PDIEDD)
  3. Drug Control and Abuse Prevention Department (DCAPD)
  4. Drug Quality Control and Toxicology Laboratory Department (DQCTLD)

Scope of Control

- Products Controlled by FMHACA
  - ✓ Human drugs
  - ✓ Radio-pharmaceuticals
  - ✓ Traditional medicines
  - ✓ Pesticides
  - ✓ Medical supplies and instruments
  - ✓ Sanitary items
  - ✓ Cosmetics
  - ✓ Raw and packaging materials
  - ✓ Food and food products

❑ Institutions Controlled by FMHACA

- ✓ Manufacturers
- ✓ Importers and/wholesalers
- ✓ Exporters
- ✓ Retail-outlets
- ✓ Drug quality control Labs
- ✓ Commission agents
- ✓ Health institutions

- **Reading Assignment**
  - EFDA: what makes it different from FMHACA?

1.3. Uncertainties in pharmaceutical analysis

- **Uncertainty** expresses the range of possible values that a measurement or result might reasonably be expected to have.
- The uncertainties encountered in any analysis give rise to three types of errors.
- **Error** is the difference between a single measurement or result and its true value.

1. *Gross errors:*

- Involve major breakdown in the analytical process and the encountered error is so serious.

➢Example:

∗Wrong dilution being prepared

∗Instruments used in the wrong way

- Give an "**outlier**" result

➢Hence there is a ***need to make a fresh*** start of the analysis

2. *Determinate/ Systematic Errors / Bias*

- Errors which can be avoided or whose magnitude can be determined
- It can be determinable and presumably can be either avoided or corrected
- It affect the accuracy

- The most common causes are:
  - ***Personal errors:***
  - e.g. Technical in competency – not following the prescribed procedure, unable to judge color change
  - ***Instrumental errors:***
  - e.g. Faulty and uncaliberated instruments

- ***Reagent errors:***
  - e.g. impurity in the reagent or expired reagent solution etc
- ***Methodological errors:***
  - e.g. failure for the reaction to proceed with the analyte, decomposition problem, improper (incorrect) sampling etc

- ***Systemic error can be:***
  - ***Additive error:*** the magnitude is independent of the sample size
  - It cause a constant absolute error in a serious of determination of increasing sample weight

  - **Proportional errors**
  - depends on the amount of the constituent
  - e.g. impurities in standard compound

Minimizing systematic errors

- Calibration of Instruments, Apparatus and Applying Necessary Corrections
- Performing a Parallel Control Determination
- Blank Determination
- Cross-checking Results by Different Methods of Analysis
- Method of Standard Addition
- Method of Internal Standards

3. *Indeterminate or Random errors*

- As the name suggests, indeterminate errors cannot be assigned to any specific and well-defined reasons.
- These errors are mostly random in nature and ultimately give rise to high as well as low results with equal probability
  - Affect the ***precision*** of the analytical experiments
  - They can neither be corrected nor eliminated
  - therefore, put the 'ultimate limitation' on the specific measurements

Assignment -1

- Method Development and validation Protocol
  - Importance
  - Parameters
    - Linearity
    - Accuracy
    - Precision
    - Sensitivity
    - Selectivity
    - LOD
    - LOQ
    - Robustness

1.4. Basic calculations in pharmaceutical analysis

Basics and definitions

- ***A solution:***
  - Is a homogeneous mixture composed of two or more substances.
    - In such a mixture, a solute is dissolved in another substance, known as a solvent.
- ***Concentrations:***
  - Is a measure how much of given substance there is mixed with another substance.

- ***Standard solution:***
  - Is a chemical term which describes a solution of known concentration.
  - The concentration of the solution is normally expressed in units of **moles per Liter** (mol/L, often abbreviated to M for molarity).
  - Standard solutions are normally used in titrations to determine the concentration of a substance in solution.

Basic calculations in pharmaceutical analysis

- There are various units used to express amounts and concentrations in pharmaceutical analysis

a) **Percentage volume/volume (%v/v)**

- is most often encountered in relation to the composition of mobile phases used in HPLC.
- Thus, when 30 ml of methanol is mixed with 70 ml of water, a 30:70 v/v mixture is formed.
- Some chromatographers prefer to prepare mixtures of solvents by weighing them rather than by volume measurement; **Why?**
  - in this case the solvent mixture can be expressed as % weight in weight (% w/w)

b) Percentage weight in volume (%w/v)

- is normally used to express the content of active ingredient in liquid formulations such as injections, infusions and eye drops.

- The density of the solvent in this case is irrelevant.
  - Eg. 1g/100 ml solution of a drug is 1%w/v whether it is dissolved in ethanol or water.

Practices

- Convert the following concentrations to % w/v:

  (i) 0.1 g/100 ml

  (ii) 1 mg/ml

  (iii) 0.1 g/ml

  (iv) 100 mg/ml

Answers:

(i) 0.1% w/v
(ii) 0.1% w/v
(iii) 10% w/v
(iv) 0.01% w/v

- **Dilutions**

  - In order for an extract from a formulation or a solution of a **pure drug substance to be measured,**

  - it must be diluted so that **it falls within the working range** of the instrument used to make the measurement.

  - Thus an understanding of **dilution factors** is fundamental to calculations based on analytical data.

- **Practices:**

1) A 2 ml volume of eyedrops containing the local anaesthetic proxymetacaine. HCl is diluted to 100 ml and then 5 ml of the dilution is diluted to 200 ml. The diluted sample was measured by UV spectrophotometry and was found to contain 0.512 mg/100 ml of the drug.

- **Calculate the %w/v** of the drug in the eyedrops.

- Dilution factors 2 to 100 (50), 5 to 200 (40). Total dilution 40 x 50 = 2000.
- Original concentration =2000x 0.512 = 1024 mg/100 ml = 1.024 g/100 ml = 1.024% w/v.

Dilution Cont...

2) A 5 ml sample of an injection of a steroid phosphate was diluted to 100 ml. Then 10 ml of the diluted injection was diluted to 100 ml and this dilution was further diluted 10 to100 ml. From measurement by UV the diluted sample was found to contain 0.249 mg/100 ml of the steroid.

What was the original concentration of the injection in **%w/v** and in **mg/ml**?

Dilution factors 2 to 100 (× 50), 5 to 200 (× 40). Total dilution 40 × 50 = 2000. Original concentration = 2000 × 0.512 = 1024 mg/100 ml = 1.024 g/100 ml = 1.024% w/v.

c) Percentage weight/weight (%w/w)

- is a common measure used to express the concentration of active ingredient in a formulation such as a cream
  - Thus, a cream containing 10 mg (0.01 g) of drug per gram is a: (0.01/1) x 100 = 1% w/w formulation.

- Used to express the content of a minor impurity in a drug substance.
  - Similarly, if a drug contains 0.5 mg (0.0005 g) per gram of an impurity, the impurity is at a concentration of (0.0005/1) x 100 = 0.05% w/w.

d) Parts per million (ppm)

- Parts per million (ppm) on a w/w basis is 1 µg/g (1 mg/kg).
- It is a common measure used for impurities in drug substances, particularly heavy metals and solvents.
- 1 ppm is also 0.000001%w/w.

- **Practice:**

- The potassium content of an intravenous infusion containing sodium chloride was determined. The infusion was found to contain 0.9092% w/v NaCl. The undiluted infusion was measured for potassium content **in comparison with a potassium chloride standard**. The potassium content of the undiluted infusion was found to be 0.141 mg/100 ml.
- Calculate the potassium content in the sodium chloride in **ppm**:

  - 0.9092% w /v = 0.9092 g/ 100 ml
  - Potassium content = 0.141mg /100 ml = 141µg /100 ml
  - Relative to the sodium chloride, there are 141 *µg/0.9092 g = 141/0.9092 ppm = 155 ppm.*

Molarity and Normality

- ***A molar solution:***
  - Is one that contains one mole of solute (molecular weight) per liter of solution.
  - If one liter of a solution contain 98 g of H_2SO4, this means that the concentration is 1M
  - If one liter of a solution contain 98x2= 196 g of H_2SO4, this means that the concentration is 2M

- ***Normal solution:***
  - Is one that contains one equivalent weights of solute per liter of solution.
  - A five normal solution of aqueous hydrochloric acid (written as "5N HCl (aq)") means there are 5 equivalents of HCl per liter of solution

$$\text{Normality} = \frac{\text{number of equivalent weights of solute}}{\text{liter of solution}} = \frac{\text{no. eq}}{\text{L}} = \frac{\text{no. meq}}{\text{mL}}$$

- **For acid:**

$$\text{To calculate the equivalent weights of acid} = \frac{\text{The molecular weight}}{\text{Number of H ions}}$$

Eg. The equivalent weights of HCl is 36.5/1 = 36.5,

The equivalent weights of H_2SO_4 is 98/2 = 49

- **For base:**

$$\text{To calculate the equivalent weights of base} = \frac{\text{The molecular weight}}{\text{number of OH ions}}$$

Eg. The equivalent weights of NaOH is 40/1 = 40,

The equivalent weights of Al(OH)3 is 78/3 = 26

Relationship exists between normality and molarity:

$$N = n \times M$$

Practice:

- How many gram of H_2SO_4 is required to prepare 10 N H_2SO_4 (aq) in 500 mL solution? (Molecular weight of H_2SO_4 is 98g/mole)

- **Given:**
  - N = 10
  - V = 500 mL =0.5 L
- **Required:**
- Actual mass?

Solution:

N = n(M); H_2SO_4 is diprotonic acid

10 = 2*M

M = **5**

M = mole/L

mole = 5*0.5 = **2.5**

mole = mass/Mwt.

Mass = 2.5*98 = **245 g**

Therefore, 245 g of H_2SO_4 is required to prepare the intended concentration.

1.5. Physical and chemical properties of drug molecules

- Physico-chemical properties of drug molecules that influence the **development of analytical methods:**
  - pH /pKa
  - Partition coefficient
  - Stability
  - Stereochemistry of the drug

Calculation of pH value of aqueous solutions

- **pH**: is a logarithmic scale which refers to the degree of acidity or alkalinity.
- It measures the concentration of H ion
  - pH of 0 means the concentration of H ion is 1 M.
  - **$pH = -\log [H^+]$**
  - Usually extends from 0 to 14.
- If the pH of an aqueous solution is known, the $[H^+]$ is given by the expression 10^{-pH}

- **Dissociation of water**

➢ In pure water the concentration of hydrogen ions is governed by the equilibrium:

$$H_2O \underset{Ka}{\rightleftharpoons} H^+ + HO^-$$

Ka *is the dissociation constant for the equilibrium, and determined by:*

$$K_w = \frac{[H^+][HO^-]}{[H_2O]} = [H^+][HO^-] = 10^{-14}$$

$$[H^+] = [HO^-] = 10^{-7}$$

The pH of water is thus given by −log 10−7 = 7.00.

Strong acids and bases

- A strong acid is completely ionized in water and $[H^+]$ is equal to its molarity,
- e.g. 0.1 M HCl contains 0.1 M H^+ (10^{-1} H^+) and has a pH of -log 0.1=1.
- For a solution of a strong base such as 0.1 M NaOH, [OH] = 0.1 M and $[0.1][H^+]=10^{-14}$;
  - therefore, $[H^+]=10^{-13}$ M and the pH of the solution=13.

- **Practices**

- Calculate the pH of the following solutions:
  - (i) 0.05 M HCl
  - (ii) 50 mM NaOH
  - (iii) 0.05 M H_2SO_4

Answers:

(i) 1.3;
(ii) 12.7;
(iii) 1.0,

Weak acids and bases

- Most drugs are either weak acids or weak bases.
- Weak acids are not completely ionized in aqueous solution and are in equilibrium with the undissociated acid.
- *Ka is given by the expression of :*

$$HA \underset{Ka}{\rightleftharpoons} A^- + H^+$$

$$Ka = \frac{[A^-][H^+]}{[HA]}$$

➢For instance, in a 0.1 M solution of acetic acid **(Ka=1.75 x10^{-5})**, *the equilibrium* can be written as follows:

$$\underset{(0.1-x)}{COH_3COOH} \overset{Ka}{\rightleftharpoons} \underset{x}{CH_3COO^-} + \underset{x}{H^+}$$

$$Ka = \frac{[CH_3COO^-][H^+]}{[CH_3COOH]}$$

$$1.75 \times 10^{-5} = \frac{x^2}{(0.1 - x)}$$

$$1.75 \times 10^{-5} = \frac{x^2}{0.1}$$

$$x = [H^+] = \sqrt{1.75 \times 10^{-6}} = 0.00132\ M$$

$$pH = 2.9$$

- The calculation of the pH of a weak base can be considered in the same way.
- For instance, in a 0.1 M solution of ammonia (Kb=1.8 x10^{-5}), the equilibrium can be written as follows:

$$\underset{(0.1-x)}{NH_3} + H_2O \overset{Kb}{\rightleftharpoons} \underset{x}{NH_4^+} + \underset{x}{HO^-}$$

$$Kb = \frac{[NH_4^+]\,[HO^-]}{[NH_3]}$$

$$1.8 \times 10^{-5} = \frac{x^2}{(0.1 - x)}$$

$$x = [HO^-] = \sqrt{1.8 \times 10^{-6}} = 0.0013\ \text{M}$$

$$[H^+] = \frac{K_w}{0.0013} = \frac{10^{-14}}{0.0013} = 7.7 \times 10^{-12}\ \text{M}$$

$$\text{pH} = 11.1$$

Acidic and basic strength and pKa

- The pKa value of a compound is defined as: pKa= -log Ka.
- A pKa value can be assigned to both acids and bases.
- For an acid, the higher the $[H^+]$ the stronger the acid.
- In the case of base, the lower the $[H^+]$ the stronger the base.

✓If pKa is used as a measure of acidic or basic strength:

- for an acid, the smaller the pKa value the stronger the acid;
- for a base the larger the pKa value the stronger the base.

Henderson–Hasselbalch equation

$$Ka = \frac{[A^-][H^+]}{[HA]}$$

- Can be substituting pH for -log [H+] and pKa for -log Ka and rearranged to give:

$$pH = pKa + \log\frac{[A^-]}{[HA]}$$

- For example, when acetic acid (pKa= 4.76) is in solution at pH 4.76, the Henderson–Hasselbalch equation can be written as follows:

$$pH = 4.76 + \log\frac{[CH_3COO^-]}{[CH_3COOH]}$$

$$4.76 = 4.76 + \log\frac{[CH_3COO^-]}{[CH_3COOH]}$$

$$\log\frac{[CH_3COO^-]}{[CH_3COOH]} = 0$$

$$\frac{[CH_3COO^-]}{[CH_3COOH]} = 10^0 = 1$$

- From this relationship for acetic acid it is possible to determine the degree of ionisation of acetic acid at a given pH.
- In this case, 50 % of the acetic acid is dissociated at a given pH.

Practice:

- Determine the percentage of ionisation of acetic acid (pKa= 4.76) at:
  - (i) pH 3.76
  - (ii) pH 5.76.

Answers: (i) 9.09%; (ii) 90.0%

- For **a base**, the Henderson–Hasselbalch equation is written as:

$$BH^+ \rightleftharpoons B + H^+$$

$$pH = pKa + \log \frac{[B]}{[BH^+]}$$

➢Ammonia (pKa 9.25) is in a solution at pH 9.25, the Henderson equation:

$$9.25 = 9.25 + \log \frac{[NH_3]}{[NH_4^+]}$$

$$\log \frac{[NH_3]}{[NH_4^+]} = 0$$

$$\frac{[NH_3]}{[NH_4^+]} = 10^0 = 1$$

➢Ammonia is 50% ionised at pH 9.25.

➢ In this case, it is the protonated form of the base that is ionised, and, as the pH falls, the base becomes more ionised.

An alternative way of writing the expression giving the percentage of ionisation for an acid or base of a particular p*Ka value at a particular pH value is:*

$$\text{Acid: \% ionisation} = \frac{10^{pH-pKa}}{1+10^{pH-pKa}} \times 100$$

$$\text{Base: \% ionisation} = \frac{10^{pKa-pH}}{1+10^{pKa-pH}} \times 100$$

- **Practice**

- Calculate the percentage of ionization of ammonia (pKa 9.25) at
  - (i) pH 8.25
  - (ii) pH 10.25.

Answers:
(i) 90.9%;
(ii) (ii) 9.09%

Buffers

- It is an aqueous solution, consists a mixture of weak acids and its conjugate base.
  - resist the drastic change in pH of the system
- Buffers are used in a number of areas of analytical chemistry, such as
  - the preparation of mobile phases for chromatography
  - the extraction of drugs from aqueous solution.
- A common buffer system is the **sodium acetate/acetic acid** buffer system.
- The most effective range for a buffer is 1 pH unit either side of the pKa value of the weak acid or base used in the buffer.
  - The pKa value of acetic acid is 4.76; thus its effective buffer range is 3.76–5.76

Partition coefficient

- The lipid/water partition coefficient denotes the ratio of the concentration of a drug in two immiscible or slightly miscible phases.

- The partition coefficient for a compound (*P) can be simply defined as follows:* $$P = \frac{C_o}{C_w}$$

- *P is often quoted as a log P-value, e.g. a log P of* 1 is equivalent to *P = 10.*
  - If partitioning is carried out between equal volumes of the organic solvent and water, then ten parts of the compound will be present in the organic layer for each part present in the water layer

Practice:

- A neutral compound has a partition coefficient of 5 between ether and water. What percentage of the compound would be extracted from 10 ml of water if (i) 30 ml of ether were used to extract the compound or (ii) three 10 ml volumes of ether were used in succession to extract the compound?

- **Given:**
  - P = 5
  - V water =10
  - V ether = 30
- **Request**
  - % of extract ?

Solution:

i. Where three volumes (30 ml) of ether were used to one volume (10 ml) of water, the distribution would be 15 parts of drug in the ether layer to 1 part in the water layer.

% Extracted = (15/16)*100 % = 93.75%

ii. % extracted = 95.5 %

Drug stability

- The purpose of drug stability testing is:
  - to provide evidence on **how the quality of a drug substance or drug product changes with time** under the influence of environmental factors such as temperature, humidity and light.
- The aim of the testing is to establish the **shelf-life of a product** and recommended **storage conditions**
- The most common types of degradation which occur in pure and formulated drugs **obey zero- or first-order kinetics**

- **Self reading:**
  - **0 order, 1st order and 2nd order degradation**

End of chapter

Thank you!